Two-hybrid screening

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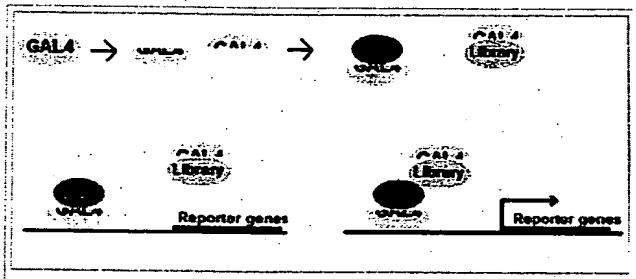
Two-hybrid screening is a molecular biology technique used to discover protein-protein interactions by testing for physical interactions (such as binding) between two proteins. One protein is termed the *bait* and the other is a *prey* or *library*.

The premise behind the test is the activation of downstream reporter gene(s) by the binding of a transcription factor onto an upstream activating sequence (UAS). For the purposes of two-hybrid screening, the transcription factor is split into two separate fragments, called Binding Domain (BD) and Activating Domain (AD). The BD is the domain responsible for binding to the UAS and the AD is the domain responsible for activation of transcription.



Overview of two-hybrid assay as follows. Binding and activating domains of transcription factor GAL4 are fused to two proteins being tested for interactions. The two proteins are termed "bait" and "library". If "bait" and "library" interact, transcription of "reporter gene" occurs. Otherwise, "reporter gene" is silent.

The key to the two-hybrid screen is that in most eukaryotic transcription factors, the activating and binding domains are modular and can function in close proximity to each other without direc binding. This means that even though the transcription factor is split into two fragments, it can still activate transcription when the two fragments are indirectly connected.

The most common screening approach is the yeast two-hybrid assay. This system utilizes a genetically engineered strain of yeast in which the biosynthesis of certain nutrients (usually amino acids or nucleic acids) is lacking. When grown on media that lacks these nutrients, the yeast fail to survive. This mutant yeast strain can be made to incorporate foreign DNA in the form of plasmids. In yeas two-hybrid screening, separate bait and prey plasmids are simultaneously introduced into the mutant yeast strain. Each plasmid contains separate genes allowing synthesis of certain nutrients. One common system utilizes bait plasmid that synthesizes the amino acid tryptophan, and the prey plasmid synthesizes the amino acid leucine. When transformed together into yeast, these plasmids allow the mutant yeast to grow on media lacking both tryptophan and leucine.

Bait plasmids are also engineered to produce a protein product in which the BD fragment is fused onto the bait protein. Prey plasmids are engineered to produce a protein product in which the AD fragment is fused onto the prey protein. Whereas the bait protein is typically a known protein that the investigator is using to identify new binding partners, the prey protein can be either a known protein or a random library protein. If the bait and prey proteins interact (i.e. bind), then the AD and BD of the transcription factor are indirectly connected and transcription of reporter gene(s) occurs. If the two proteins do not interact, there is no transcription of the reporter gene. Typically, reporter genes encode for enzymes that allow synthesis of other specific nutrients that the mutant yeast strain is otherwise unable to produce. In one system, reporter genes include histidine and adenine biosynthesis. Thus, if two proteins in a screen interact, yeast containing these proteins will grow on media lacking tryptophan (allowed by the bai plasmid), leucine (allowed by the prey plasmid), adenine and histidine (allowed by interaction between bait and prey proteins, ing reporter genes). A screen in which there is no interaction between bait and prey proteins would yield yeast that grow on ...dia lacking tryptophan and leucine only (because without bait/prey interaction, the AD and BD do not form a functional transcription factor allowing reporter nutrient biosynthesis). Another reporter gene includes an enzyme system to produce blue colc - interactions result in yeast colonies that can generate blue color under certain conditions.

With a certain bait protein, two hybrid screening can be "directed" to test for protein-protein interaction with a known protein inserted into prey plasmid. Alternatively, "library screening" involves pairing bait protein with millions of different prey plasmids that have been engineered to produce protein from a unique, randomly inserted DNA fragment. The DNA fragments in library prey plasmids are synthesized from messenger RNA from a specific organism or tissue. As such they represent a "library" of the protein expressed in a given organism or tissue and allow investigators to search for new protein-protein interactions from amongst all the

proteins expressed in the species or tissue of choice.

Related techniques include one-hybrid screening and three-hybrid screening. The former tests directly for interaction between the library protein and a DNA target and the latter includes a third protein that bridges the bait and library proteins.

A common transcription factor used for yeast two-hybrid screening is GAL4.

External links

- Detail on sister technique two-hybrid system (http://www.biochem.arizona.edu/classes/bioc568/two-hybrid_system.htm)
- Science Creative Quarterly's overview of the yeast two hybrid system (http://www.scq.ubc.ca/?p=246)

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